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Research Article

Antioxidants activities study of different parts of extracts of *Khaya senegalensis* (Desr.) A. Juss. (Meliaceae)

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Abstract

Khaya senegalensis (Desr.) A. Juss (Meliaceae) is a plant that has long been used in the Burkina pharmacopoeia for the treatment of many diseases due to its many pharmacological properties. The trunk bark has long been the target of traditional health practitioners. The aim of this study was to compare the antioxidant activity of trunk bark, leaves and fruits (hulls and seeds) in order to show whether these parts which are easily renewable could be used in place of the bark of the trunk. Aqueous and hydroalcoholic extracts have been made from powders of the bark of the trunk, leaves, hulls and seeds of *Khaya senegalensis*. A phytochemical study was carried out beforehand from these extracts and made it possible to highlight tannins, flavonoids, saponosides, coumarins, anthraquinones, emodols, triterpenes and sterols which are polyphenols endowed with antioxidant activity. Evaluation of the antioxidant effect of aqueous extracts showed a reduction in DPPH with IC_{50}s of 27.89 ± 0.07 µg / ml, 44.88 ± 0.43 µg / ml, 54.62 ± 0.87 µg / ml and 55.56 ± 0.23 µg / ml for bark, leaves, hulls and seeds, respectively. This shows that the different parts are gifted with antioxidant activity and the barks are more powerful than the others. These results show that for hydroalcoholic extracts, the leaves and fruits could be used as a replacement for the bark, but factors such as the high cost of producing these extracts, the periodicity of fruiting and the difficulty of harvesting would limit their use.

Keywords: *Khaya senegalensis,* phytochemical constituent, antioxidant activity.

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1. Introduction

In biological system, reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as superoxide, hydroxyl, and nitric oxide radicals are responsible for extensive cell damage including induction of DNA breaks and mutations, modification of protein structures, lipid peroxidation, inactivation of various enzymes and oxidation of sugars [1][2]. Oxidative/nitrosative stress represents the bodies' imbalance in the production and the elimination of reactive oxygen and nitrogen species as well as decreased production of antioxidants [3]. This oxidative stress can be at the origin of numerous pathologies such as cancer, cataracts, amyotrophic lateral sclerosis, broncho pneumopathies, respiratory insufficiencies, Alzheimer's disease, rheumatism and cardiovascular diseases [4][5]. Oxidative stress is of great interest, especially since the pathologies in which it is blamed are real public health burdens. To prevent the occurrence of pathologies linked to oxidative stress; it is therefore not irrational to think that an intake of antioxidants independent of food can be beneficial for the body. Medicinal plants are an important source of substances with very varied biological and pharmacological activities, namely polyphenols, flavonoids, carotenoids, etc [6]. These secondary metabolites are natural antioxidants which can reduce the access of oxidants and other deleterious molecules through their ability to scavenge free radicals and activate antioxidant enzymes [7]. In this context, this study focused on *Khaya senegalensis* (Meliaceae), a medicinal plant, used in Burkina Faso by traditional therapists, for the treatment of various diseases such as chronic wounds, fever and inflammation [8][9], gastrointestinal disorders [10]. The trunk and roots bark, the leaves and the fruits of *Khaya senegalensis* are the target of these traditional healers. The massive use of these different parts of the plant leads in the long term to the disappearance of the species [11][12]. Its overexploitation has earned it a classification among the protected species in Burkina Faso. Our study consists of comparison of the antioxidant properties of the leaves, trunk bark and fruits of *Khaya senegalensis* (Ders.) A. Juss. (Meliaceae) in order to establish a possibility of substituting the bark by fruits or leaves which are easily renewed by the plant.

2. Material and methods

2.1. Plant material

The plant material consisted of stem bark, leaves, hulls and seeds of *Khaya senegalensis* collected in the rural commune of Koubri, central plateau region. The harvested samples were dried under ventilation artificial shielded from light in the laboratory of the Department of Medicine - pharmacopoeia traditional / pharmacy (MEPHATRA / PH) of the Institute for Research in Health Sciences. After drying, the various parts harvested were reduced to powder using a grinder (East Gladiator 1931 Type BN 1 Mach 40461 1083) and store in a plastic bag for studies.

2.2. Extraction

Maceration (aqueous and methanolic) was performed and extraction by exhaustion with solvents of increasing gradient (petroleum ether-ethyl acetate-methanol / water: 80/20). An aqueous maceration of 50 g of each vegetable powder in 250 ml of distilled water was carried out for 24 hours, and then filtered. Each filtrate was frozen and lyophilized.

A hydroalcoholic maceration of 50 g of each vegetable powder in 250 ml of methanol / water (80/20: v / v) mixture was carried out with stirring for 24 hours, and then filtered. The filtrate was then concentrated by evaporation of the solvent.

For extraction by exhaustion, 100 g of each powder were macerated in 500 ml of petroleum ether (in order to remove all the fat and the chlorophyll) with stirring then pass through the percolator until obtaining ether of clear petroleum. On the marc, ethyl acetate is also passed under the same conditions as before. Finally, the hydroalcoholic fraction was obtained by maceration of the marc with a methanol / water mixture (80/20: v / v). Extract was concentrated by evaporation of the solvent.

2.3. Phytochemical screening

Phytochemical screening was carried out on chromatoplates (60 F250, 20x20 glass support, Fluka-Silica gel) according to the methods described in the literature [13]. This involved searching for large chemical groups by thin layer chromatography (TLC) such as terpene and sterols compounds, phenolic compounds, nitrogen compounds. Several specific reagents have been used to reveal these groups of compounds: anthocyanins and anthraquinones, alkaloids, flavonoids, tannins and saponosides.

2.4. Antioxidant activity: DPPH$^{\bullet}$ assay

DPPH$^{\bullet}$ radical scavenging activity was done according to Kim *et al*. [14]. 20 µl of extract or fractions or standard was added to 200 µl of DPPH methanolic solution (0.04 mg ml^{-1}) in a 96-well microtiter plate and vortexed. After 30 min incubation in the dark at room temperature, the absorbance was measured at 490 nm using spectrophotometer BioRAd (model 680, Japan). Each determination was carried out in triplicate. Antiradical activity was defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% and expressed as antiradical power (ARP = $1/IC_{50}$).

3. Results and discussion

3.1. The extraction yields and residual moisture

The extraction yield and residual moisture of powder are mentioned in the table 1.Residual moisture content of the various powders was between 6.63 ± 0.29 for the powder of the seeds to 9.59 ± 0.83 for the powder of the plant hulls Performed with the aqueous extract, the yields of different parts of the plant differed from 13.74±1.03 for trunk bark to 17.90±2.73 % for hulls.

Table 1: Extraction yield and residual moisture

Extracts	Stem bark	Leaves	Hulls	Seeds
residual moisture %	7.71 ± 0.24	8.72 ± 0.51	9.59 ± 0.83	6.63 ± 0.29
Extraction percentage %	13.74 ± 1.03	15.93 ± 2.11	17.90 ± 2.73	16.75 ± 2.20

These contents are all less than 10% and suggest that the extracts could be saved for the purpose of the study. The hulls presented the best extraction performance compared to other parts of the plant. This could be explained by the richness of the hulls in different compounds that are more extractable than the other parts of the plant.

3.2. Phytochemical screening

Analysis by thin layer chromatography (TLC) revealed the presence in selective extract such as hydroalcoholic and ethyl acetate fraction of the different parts of the plant studied of the chemical groups shown in Table 2. An example of a chromatogram is shown in figure 1. The compounds sought were compounds known for their antioxidant properties.

Table 2: Chemical groups in hydroalcoholic and ethyl acetate fractions of plant by TLC

Extracts	Hydroalcoholic fraction				Ethyl acetate fraction			
	Trunk bark	Leaves	Hulls	Seeds	Trunk bark	Leaves	Hulls	Seeds
Flavonoids	+	+	-	-	+	+	+	+
Tannins	+	+	-	+	+	+	-	+
Anthocyanins	+	+	-	+	+	+	+	+
Anthraquinones	+	+	-	+	+	+	+	+
Saponosides	+	+	+	+	+	+	+	+
Alkaloids	-	-	-	-	-	-	-	-

+: Presence; **-**: Absence

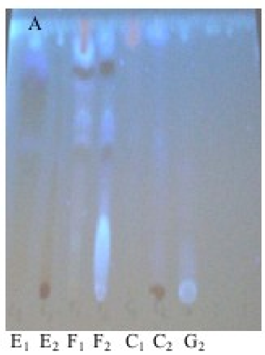

Figure 1: Chromatogram of saponosides revealed in daylight or UV; A: UV at 254 nm and B: UV after revelation

TLC screening performed on the hydroalcoholic fraction of trunk bark and of leaves revealed tannins, flavonoids, anthocyanins, anthraquinones and saponosides. With the exception of the flavonoids, the seeds contained all of the other chemical groups. The hulls of the plant contained only saponins. The TLC screening performed on the ethyl acetate fractions showed all 4 parts of the plant studied contained the flavonoids, anthocyanins, anthraquinones and saponosides. Tannins are present in all 3 parts of the plant except the hulls. Alkaloids were absent in all fractions of different parts of plant. Lompo *et al.*, [10] reported the presence of tannins in the leaves and bark of the plant. The presence of flavonoids, anthocyanins and anthraquinones has been reported in leaves and trunk bark in the work of Lompo [15], Lompo *et al.*, [10], and Atto *et al.*, [16]. The absence of alkaloids has also been noted by many authors [17][16]. The secondary metabolites identified in the fractions are suspected to be responsible for pharmacological properties [6]. Indeed, flavonoids are endowed with antioxidant properties. Tannins manifest the properties of vitamin P. Dihydroxyanthracenics are endowed with antibacterial properties[18]. As the leaves contain the same secondary metabolites as the trunk bark, they could replace the latter if, however, they possess the same antioxidant activities. The seeds could also replace the bark of the trunk although they do not contain the tannins which are of great importance in the antioxidant activity of the plants.

3.3. Antioxidant activity determinations: DPPH• assay

Study of the antioxidant activity of hydroalcoholic, aqueous extracts, hydroalcoholic and ethyl acetate fractions by the method of reduction of DPPH radical has shown that the different parts of *Khaya senegalensis* are all endowed with antioxidant properties at high levels varying concentrations. The results obtained are shown in Table 3.

Table 3: Antioxidant activities of the various extracts of *K. senegalensis*

Extracts	Aqueous	Hydroalcoholic	Ethyl acetate fraction	Hydroalcoholic fraction
Trunk bark	27.89±0.07	47.14±1.22	45.64±0.67	73.23±0.73
Leaves	44.83±0.43	34.52±0.64	31.03±0.89	76.64±0.93
Coques	54.62±62	15.61±0.24	49.35±0.74	13.38±0.88
Seeds	55.56±0.23	11.54±0.93	-	78.74±0.37
Quercetin			3.85±0.02	

The aqueous and hydroalcoholic extracts of the trunk bark, leaves, hulls and seeds of the fruit of *Khaya senegalensis* and quercetin (control) caused a reduction of the DPPH radical in vitro. This effect increased with dose of the extract. The 50 percent inhibitory concentrations (IC_{50}) of the aqueous extracts ranged between 27.89 ± 0.07 µg / ml, and 55.56 ± 0.23 µg / ml, respectively for trunk bark and seeds (Figure 2 A). However, the aqueous extract of the bark is more active than the other extracts but remains less active than quercetin which is a pure compound. Trunk bark is more active than the leaves which were more active than the hulls and seeds. Karou [19] reported that the trunk bark of *Khaya senegalensis* had the highest content of phenolic compounds than the leaves. This study applied to hydroalcoholic extracts and to quercetin had reported that the seeds and hulls are more active than the bark (47.14 ± 1.22 µg / ml) and the leaves (34, 52 ± 0.64 µg / ml) (figure 2B) with respectively 11.54 ± 0.93 µg / ml and 15.61 ± 0.24 µg / ml but remain less active than quercetin (3.85 ± 0.02 µg / ml). For therapeutic use of this extract it is preferable to use the husks or seeds because of their strong antioxidant power.

For the hydroalcoholic extracts obtained after exhaustion, a reduction of the DPPH radical was also observed (Figure 2 D). This activity is variable depending on the parties. The IC_{50}s were 13.38 ± 0.88 µg / ml, 73.23 ± 0.73 µg / ml, 76.64 ± 0.93 µg / ml and 78.74 ± 0.37 µg / ml respectively for the hulls, bark, leaves and seeds (Table 2).

The hulls are more potent than the bark, leaves and seeds but less potent than quercetin (Figure 2 C). At the level of the ethyl acetate extract, the reduction of the DPPH radical made it possible to evaluate greater IC_{50}s, i.e. 31.03 ± 0.89 µg / ml for the leaves, bark and hulls gave Nearby IC_{50} in order of 45.64 ± 0.67 µg / ml and 49.35 ± 0.74 µg / ml respectively (Table II). The antioxidant effect of the leaves is more potent than the bark and cockles and less potent than quercetin. If we consider the hydroalcoholic extracts, the hulls and seeds of the fruit are more interesting as an antioxidant while the leaves are better for the extracts of ethyl acetate.

The phosphomolybdate reduction method applied to polyphenols has shown good activity of the bark and leaves of *Khaya senegalensis* [19][20]. Hence the existence of a good correlation between the contents of polyphenols and the antioxidant activity. Numerous studies have reported that polyphenols and flavonoids are potential reducing agents for the DPPH radical [6]. Indeed, the redox properties of these phenolic compounds would allow them to act as reducing agents, hydrogen donors, singlet oxygen deactivators and also metal chelators [21]. More stable tannic radicals would then be formed, which would have the consequence of stopping the chain reaction of the autooxidation of lipids [22]. Regarding flavonoids, because of their low redox potentials, they would be able to reduce oxidizing free radicals such as superoxide, peroxyl, alkoxyl and hydroxyl, by transfer of hydrogen and the flavonoxy radical which in result could react with another radical to form a stable structure [23]. They would also be able to trap metal ions, because they have chelating properties [24]. This link between phenolic compounds and antioxidant activity could explain the use of the plant.

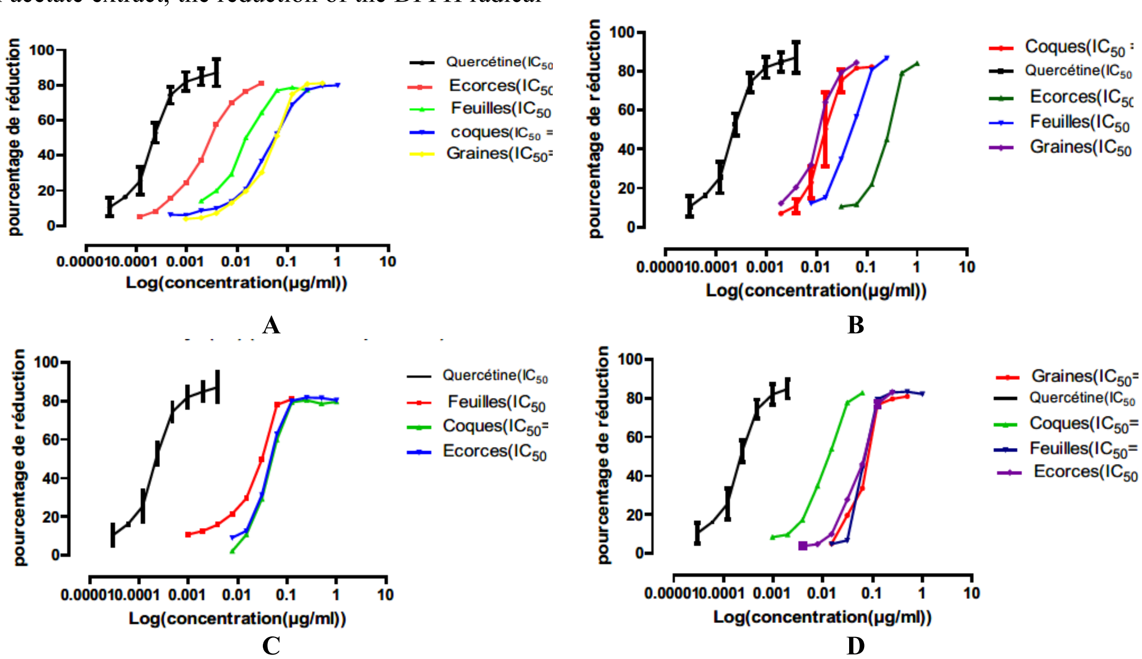

Figure 2: Percentage reduction of DPPH radicals as a function of the concentrations of the aqueous extracts (A), hydroalcoholic (B), ethyl acetate fraction (C) and hydroalcoholic fraction (D) of trunk bark, leaves, hulls and fruits of *Khaya senegalensis* and quercetin.

4. Conclusion

The present study has made it possible to demonstrate by scientific tests the presence of tannins, flavonoids, saponosides, anthocyanins and anthraquinones in the aqueous and hydroalcoholic extracts of trunk bark, leaves, hulls and seeds of *Khaya senengalensis* as well as the antioxidant activity of the various extracts. The compounds highlighted in the different extracts are responsible for their antioxidant properties. In view of our results, the trunk bark is preferable to other parts of the plant for use of the plant for optimal treatment of pathologies involving oxidative stress.

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